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^{1}H NMR Spectroscopy of the Hydrogen-Bonded Imide Groups of Hub(M)$_3$:3CA Provides a Useful Method for the Characterization of These Aggregates

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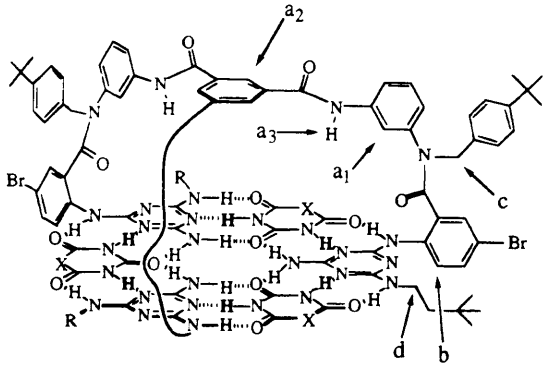
Variable-temperature ^{1}H NMR spectroscopy of the region between 13 and 16 ppm can be used in the conformational analysis of hydrogen-bonded aggregates comprising 1 equiv of a trismelamine (hub(M)$_3$) and 3 equiv of an isocyanuric acid (CA). Two types of isomers are observed: a symmetric C_3 isomer (identified by two lines in this spectral region at low temperature) and an asymmetric C_1 isomer (identified by six lines at low temperature), each existing as a pair of enantiomers. The isomers have similar structures: the N—H connectivity established by nuclear Overhauser effects are consistent with a rosette motif. The relative concentrations of isomers depend slightly on solvent (CD_2Cl_2, $CDCl_3$, $C_2D_2Cl_4$) and on the structure of the CA (barbital, dibromobarbituric acid, triphenylpropylisocyanuric acid, and neohexylisocyanuric acid); the C_3 isomer is favored over the C_1 isomer by ca. 0.7 kcal/mol in all cases. This N—*H* region of the NMR spectrum also carries information about the dynamic behavior of these aggregates. The exchange of hydrogen-bonded imide protons between different environments leads to the coalescence of pairs of imide lines. This exchange-mediated coalescence occurs at different temperatures and allows (with additional NOE data) individual CA groups to be identified. The activation energy for the exchange process or processes leading to coalescence of pairs of imide lines is ~14 kcal/mol. This process is faster than the interconversion between C_3 and C_1 isomers.

Introduction

Analysis of the ^{1}H NMR spectrum of hydrogen-bonded imide N—*H* groups (in the region between 16 and 13 ppm) of aggregates of the structure hub(M)$_3$:3CA (Scheme 1)[3] provides thermodynamic and kinetic information useful in characterizing these aggregates. The simplicity of this region of the spectra often allows the complete assignment of peaks; the higher field region of the proton spectrum is complicated and does not provide reliable signatures of different types of structures. Counting N—H peaks at low temperatures establishes the number and symmetry of the isomers of hub(M)$_3$:3CA and equilibrium constants and differences in free energy between them. Variable-temperature (VT) NMR studies reveal the relative rates of certain dynamic processes taking place, including interconversion between the C_3 and C_1 isomers, and processes leading to the coalescence of pairs of imide lines.

Hub(M)$_3$:3CA as a Model System. Hub(M)$_3$:3CA is an aggregate based on the melamine·isocyanuric acid rosette (M_3·CA_3) comprising four molecules: one trismelamine, hub(M)$_3$, and three molecules of CA.[4] The aggregate is stabilized by 18 hydrogen bonds. Hub(M)$_3$:3CA is a relatively simple system: it has a tractable number of different types of protons, and it exists as only two conformational isomers (each with an enantiomer that is indistinguishable in the nonchiral medium provided by the solvents surveyed in this paper).[5] An additional advantage in using the hub(M)$_3$:3CA system is that a series of related aggregates can be generated for examination efficiently by simply substituting different isocyanuric and barbituric acids (CAs) into the aggregate. The CAs used in this study are shown in Scheme 1.

Scheme 1. The Hub(M)$_3$:3CA Aggregate Is Based on the Cyanuric Acid·Melamine Rosette[a]



[a] The aggregates (**1**, X = CEt_2; **2**, X = CBr_2; **3**, X = N-$(CH_2)_2C(Ph)_3$; **4**, X = $N(CH_2)_2C(CH_3)_3$) studied are generated by substituting different molecules of CA (barbital, dibromobarbituric acid, triphenylpropylisocyanuric acid, neohexylisocyanuric acid). R = neohexyl in this scheme. All three linkers connecting the hub with the melamines are the same; the one in front of the rosette is indicated schematically as a wavy line. The letters correspond to the NMR spectra shown in Figure 3. The imide protons are shown in bold.

It has not been possible to grow crystals and determine

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(1) M.I.M.W. acknowledges King Fahd University of Petroleum and Minerals, Saudia Arabia for a sabbatical year at Harvard.
(2) J.P.M. was a SERC/NATO Postdoctoral Fellow, 1991–1993.
(3) For simplicity, we will refer to both isocyanuric acids and barbituric acids as "CA".
(4) Seto, C. T.; Whitesides, G. M. *J. Am. Chem. Soc.* **1990**, *112*, 6409–6410. Seto, C. T.; Whitesides, G. M. *J. Am. Chem. Soc.* **1991**, *113*, 712–713. Seto, C. T.; Whitesides, G. M. *J. Am. Chem. Soc.* **1993**, *115*, 1330–1340.
(5) We are currently pursuing these issues with chiral shift reagents and optically active isocyanurates.

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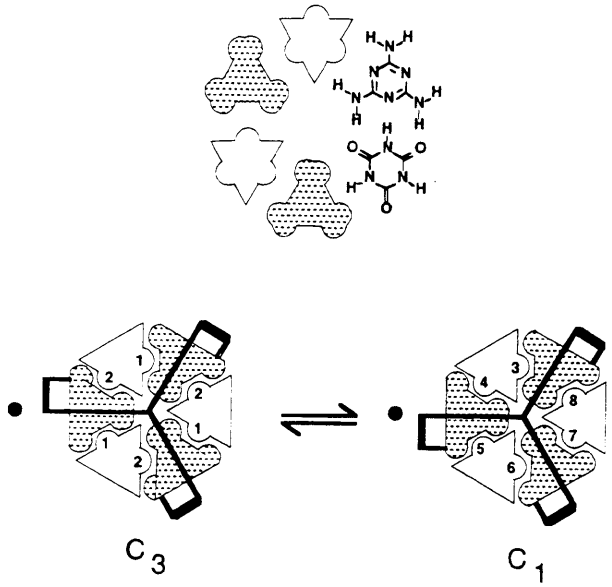
Scheme 2. The Rosette Is a Hydrogen-Bonded Aggregate That Alternates Isocyanuric Acids (White) and Melamines (Grey), and Hub(M)$_3$:3CA Is Able To Adopt Two Configurations (Based on the Orientation of the Spoke ·).



[a] The C_3 isomer and C_1 isomer are shown (enantiomers have been omitted). The hydrogen-bonded imide protons numbered) appear between 13 and 16 ppm in the ^{1}H NMR spectrum.

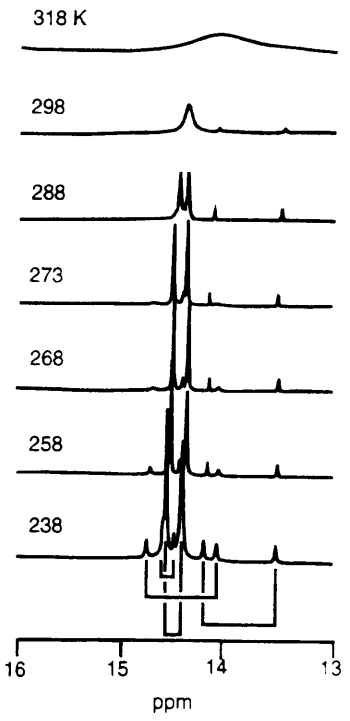


Figure 1. ^{1}H NMR spectra of 1 (X = CEt$_2$) in CD$_2$Cl$_2$. The resonances of the C_3 and C_1 isomers are indicated beneath the spectrum.

the structures of Hub(M)$_3$:3CA or others of these aggregates by single-crystal X-ray diffraction, although a related structure of a cyclic rosette is available.[6] As a consequence, structural assignments have been based on information from ^{1}H NMR spectroscopy, gel permeation chromatography (GPC), vapor-phase osmometry (VPO), and UV spectroscopy. As the structures of these aggregates get larger and more complex, the ^{1}H NMR spectra also become more complex. The resonances for the imide N—*H* protons of the CA equivalents involved in hydrogen bonding are the only peaks that appear in the region of the ^{1}H NMR spectrum between 13 and 16 ppm. The simplicity of this region of the spectrum makes it particularly useful as a source of readily interpretable information about a series of these aggregates. The objective of this study was to explore the spectroscopic behavior of one set of aggregates in detail, both to define structures for these aggregates and to establish the methodology for using this region of the NMR spectrum for structural analysis of them.

Hub(M)$_3$:3CA Can Exist as a Symmetric Isomer or an Asymmetric Isomer. Hub(M)$_3$:3CA aggregates can, in principle, exist in two distinct conformations: a symmetric isomer (C_3) and an asymmetric isomer (C_1) with enantiomers of each (Scheme 2). Symmetry dictates that only two types of imide protons exist in the C_3 isomer: N—*H* protons far from the spoke—ring junction of the adjacent melamine and N—*H* protons close to a spoke—ring junction of the adjacent melamine. These protons are label as "1" and "2" in Scheme 2. The C_1 isomer has no symmetry equivalent hydrogens, and the ^{1}H NMR spectrum can, in principle, show six separate peaks labeled "3"—"8". A 1:1 mixture of the C_3 and C_1 isomers would be predicted to show two singlets of intensity 3 and six singlets of intensity 1 if all the imide protons were nonequivalent and underwent slow exchange on the NMR time scale.

Results

Aggregate 1 Exists as a Mixture of the C_3 and C_1 Isomers. We previously reported that the NMR spectrum of 1 showed two major resonances in the region of the ^{1}H NMR spectrum between 13 and 16 ppm and that, in addition, minor resonances were observed depending on the isocyanate or barbiturate used.[4] Examination of 1 in CD_2Cl_2 over the temperature range from −55 to 50 °C reveals the pattern of eight lines expected for a mixture of distinguishable C_3 and C_1 isomers (Figure 1). At −40 °C and lower, the six resonances of the C_1 isomer have the same intensity, as do the two resonances of the C_3 isomer. Aggregate 1 also exists as the C_3 and C_1 isomers in $CDCl_3$ and $C_2D_2Cl_4$ (Figure 2). In $C_2D_2Cl_4$ not all of the resonances of the C_1 isomer are resolved before increased viscosity leads to spectral broadening.

Evidence for the C_1 Isomer is Present in the Upfield Region of the Spectrum (Figure 3). The three nonequivalent spokes of the C_1 isomer are expected to generate three lines in the ^{1}H NMR spectrum for each line of the C_3 isomer. Some of these resonances in the upfield region of the spectra are readily distinguishable. In these indicated cases, the three peaks of the C_1 isomer have equal areas, and also show chemical shifts and coupling constants similar to those of the C_3 isomer.

The Imide Protons of Specific Isocyanurates Can Be Assigned Using NOE Data and VT ^{1}H NMR Spectroscopy. As a result of symmetry, the C_3 isomer has only one type of CA site; each molecule of CA has two different imide N—*H* protons. We can assign these protons on the basis of NOE connectivity. The upfield resonance of the pair shows an NOE to an N—*H* proton

(6) Zerkowski, J. A.; Seto, C. T.; Whitesides, G. M. *J. Am. Chem. Soc.* **1992**, *114*, 5473–5475.

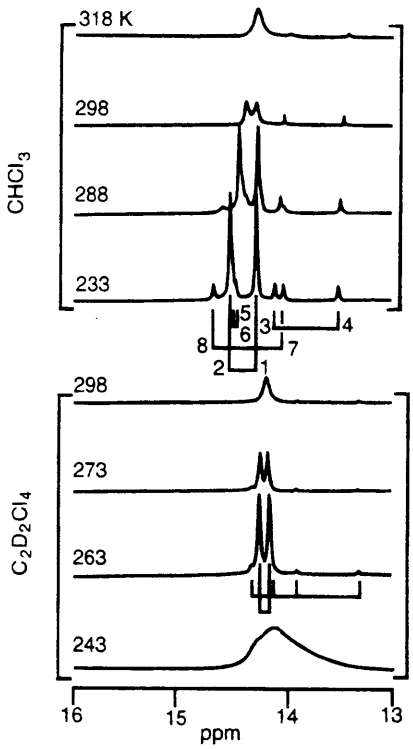


Figure 2. ^{1}H NMR spectra of **1** in $CDCl_3$ and $C_2D_2Cl_4$. The resonances of the C_3 and C_1 isomers are indicated beneath the spectrum. The numbers appearing on the spectra recorded in $CDCl_3$ refer the imide protons indicated in Scheme 2. Assignment is based on the pairwise appearance of peaks (identified with brackets) and NOE data.

of the neohexylamino group (found on the adjacent melamine) and therefore, this resonance corresponds to proton "1" of Scheme 2. Similarly, the downfield resonance of the pair shows an NOE to the N—*H* proton of the *p*-bromoanthranilate group (of the other adjacent melamine) and therefore, this resonance corresponds to proton "2" of Scheme 2.

To assign protons "3"—"8" of the C_1 isomer, we group the six lines into three pairs (corresponding to the three different molecules of CA) and catagorize each line (by NOE) as close to either the N—*H* of an anthranilate or the N—H of a neohexylamino group. The VT ^{1}H NMR spectra offers a means with which to group the resonances arising from the same CA into pairs. As the temperature is lowered, both imide protons of a single CA appear simultaneously. For example, the first and third (starting upfield and counting downfield) lines of Figure 2 (in $CDCl_3$) appear together at 318 K and belong to one of the three molecules of CA of the C_1 isomer. Fortunately, the appearance of pairs of imide resonances occurs at different temperatures for different CA groups. The identification of these pairs is indicated in Figure 2 ($CDCl_3$) with brackets.

To complete the assignment of the imide lines of the C_1 isomer, a defining NOE (to the N—*H* proton of the *p*-bromoanthranilate group or N—*H* proton of the neohexylamino group) is necessary. These NOEs were obtained, and the complete assignment appears in Figure 2. Both the first and third lines (one pair) show NOEs to N—*H* proton of a neohexylamino group. These lines correspond, therefore, to those protons labeled "3" and "4" in Scheme 2; although we cannot differentiate between the two on the basis of this information. Similarly,

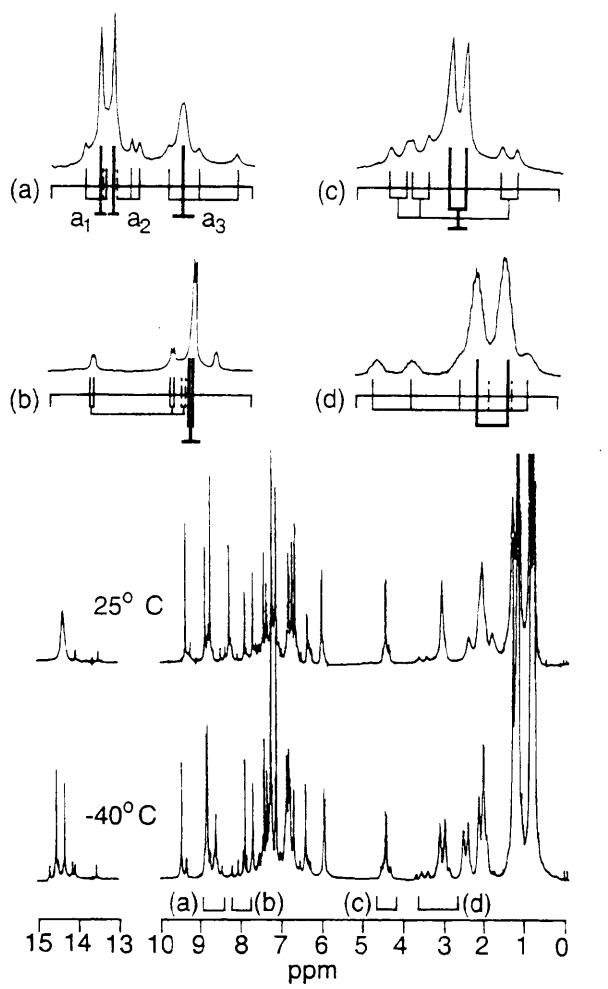


Figure 3. The entire spectra of **1** in $CDCl_3$ recorded at 25 °C and −40 °C are shown. Each resonance of the C_3 isomer appears three times for the C_1 isomer in the low-temperature spectrum (bottom). Regions where this tripling of resonances is readily observable are indicated with brackets and letters under the low-temperature spectrum. These regions are expanded (above); lines of the C_3 isomer are shown darker and the lines of the C_1 isomer are lighter and connected with brackets. In cases where resonances overlap substantially, a dashed line is used to indicate the presence of the obscured resonance of the C_1 isomer. The protons responsible for the lines of the expanded regions are identified in Scheme 1.

the second and last lines appear simultaneously at 288 K. The second line shows an NOE to the N—*H* proton of a neohexylamino group, while the last line shows an NOE to the N—*H* proton of the *p*-bromoanthranilate group. We conclude that these must be protons "7" and "8" respectively. The remaining two lines both show NOEs to the N—*H* protons of the *p*-bromoanthranilate groups and correspond to protons "5" and "6"; although again, we cannot distinguish these two with the information available.

Aggregates 2 and 3 Also Exist as a Mixture of C_3 and C_1 Isomers in CD_2Cl_2, $CDCl_3$, and $C_2D_2Cl_4$. Figures 4 and 5 include representative spectra from each analysis. Some of the resonances of the C_1 isomer of **2** are difficult to identify at low temperatures in CD_2Cl_2 and $C_2D_2Cl_4$.

Aggregate 4 Exists Primarily as the C_3 Isomer. The Anomalous Spectra of 4 in Chloroform Seem To Be Due to a Second C_3 Isomer. The variable-temperature spectra of **4** are anomalous (Figure 6). As

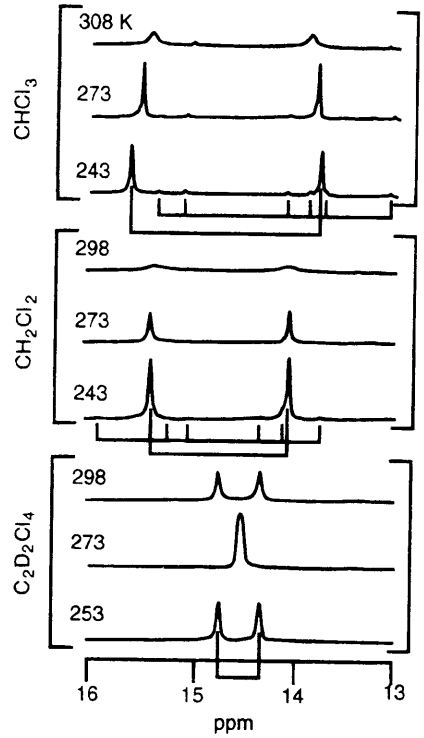


Figure 4. ^{1}H NMR spectra of **2** (X = CBr_2) in CD_2Cl_2, $CDCl_3$, and $C_2D_2Cl_4$. The resonances of the C_3 and C_1 isomers are indicated beneath the spectrum.

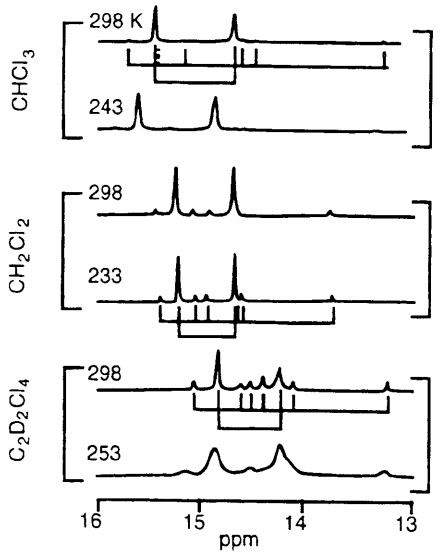


Figure 5. ^{1}H NMR spectra of **3** [X = $N(CH_2)_2C(Ph)_3$] in CD_2Cl_2, $CDCl_3$, and $C_2D_2Cl_4$. The resonances of the C_3 and C_1 isomers are indicated beneath the spectrum.

the temperature is lowered, no peaks corresponding to a C_1 isomer appear (the detection limit of the instrument is approximately 1%). We infer that the C_3 isomer is more stable than the C_1 isomer by at least 2 kcal/mol. In chloroform, as the temperature is lowered, a second set of resonances appears. These resonances correspond to what we believe is a second C_3 isomer. We hypothesize that this isomer is one that encapsulates a molecule of solvent within the central hole of the aggregate. A related phenomena has been reported by Rebek.[7] The appearance of additional resonances (of the correct

(7) Branda, N.; Wyler, R.; Rebek, J. *Science* **1994**, *263*, 1267.

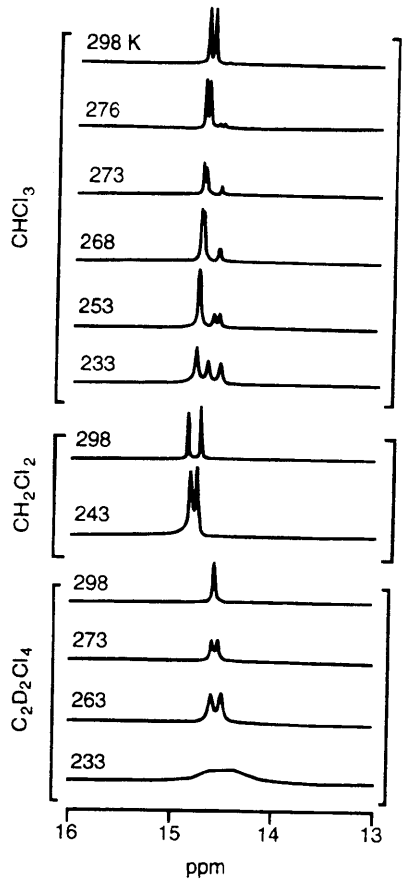


Figure 6. ^{1}H NMR spectra of **4** [X = $N(CH_2)_2C(CH_3)_3$] in CD_2Cl_2, $CDCl_3$, and $C_2D_2Cl_4$.

Table 1. Values of K_{eq} (and ΔG, kcal/mol) for the C_3 and C_1 isomers[a]

solvent X =	1 CEt_2	2 CBr_2	3 $N(CH_2)_2C(Ph)_3$	4 $N(CH_2)_2C(CH_3)_3$
CD_2Cl_2	10 (1.1)[b]	29 (1.6)[b]	12 (1.2)[b]	300 (>2.6)[c]
$CDCl_3$	8 (1.0)[b,d]	12 (1.2)[b]	21 (1.4)[b]	300 (>2.6)[c]
$C_2D_2Cl_4$	26 (1.7)[e]	300 (>2.6)[c]	5 (0.7)[e]	300 (>2.6)[c]

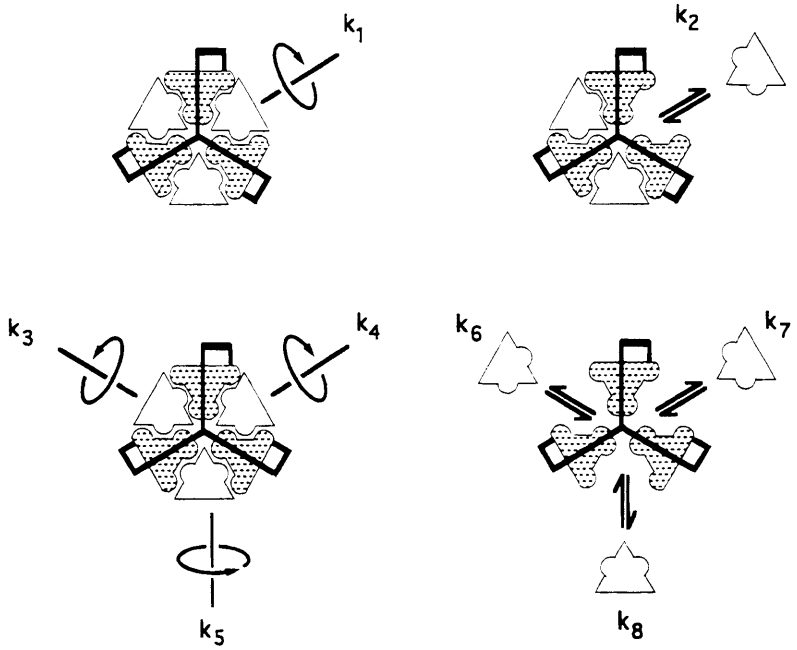
[a] The values were obtained by integrating the spectra at low temperature: the area under the lines corresponding to the C_3 isomer was divided by the 1/3 the area under the lines of the C_1 isomer. See text and note 9 for details. [b] Calculated at T = 233 K. [c] Although no lines were observed we estimate the lower limit on the basis of the 1% appearance of the C_1 isomer and T = 233 K. [d] Measurements taken in a chloroform solution that was presaturated with water gave the indistinguishable results. [e] Calculated at T = 263 K.

chemical shift and intensity) in the upfield region of the spectra is consistent with the assertion that a second isomer is present. NOE studies also suggest that this new aggregate is of similar structure to the original C_3 isomer. We will report a detailed study of the connection between the size and structure of solvent and the appearance of this second C_3 isomer after further examination.[8]

Values for the Equilibrium Constant (K_{eq}) and Difference in Free Energy (ΔG) for the Conformational Isomers Can Be Calculated from the Low-Temperature NMR Spectra. Table 1 shows the values of K_{eq} and ΔG for the aggregates **1**–**4** in three solvents for the interconversion described in eq 1. These values are corrected for the difference in symmetry between the

(8) Simanek, E. E.; Whitesides, G. M. Unpublished results.

Scheme 3. The Processes Leading to the Coalescence of Pairs of Imide Protons Are Shown[a]



[a] (Top) Two processes can lead to coalescence in the C_3 isomer: rotation of a molecule of CA while complexed (k_1) and dissociation/reassociation (k_2). (Bottom) The picture is the same for the C_1 isomer, although extended due to the presence of different environments. Rotation of a CA leads to three different rate constants (k_3, k_4, and k_5). Dissociation/reassociation also leads to three different rate constants (k_6, k_7, and k_8). See the text for details of the "environments".

C_1 and C_3 isomers.[9]

$$K_{eq} = {}^1/_3[C_1]/[C_3] \quad (1)$$

Discussion

Aggregates Comprising Isocyanurates Are More Stable than Aggregates Comprising Barbiturates. All resonances corresponding to the C_1 and C_3 isomers are resolved at room temperature for **3**. Only at lower temperatures are the corresponding resonances of **1** and **2** resolved. From this observation, we conclude that exchange of CA groups between hydrogen-bonding sites is slower for isocyanurates than for barbiturates. Aggregates comprising isocyanurates, therefore, are more stable than aggregates comprising barbiturates. It is unclear, from the present data, why there is a difference.[10] CPK models do not indicate that there are any regions in which differences in the extent of van der Waals interactions could favor one type of CA over another. The values[11] of the pK_as of the imide protons (for **1**, 8.4; **2**, 5.9; **4**, 9.0) do not vary in a manner consistent with the trend in stability. The only qualitative difference between isocyanurates and barbiturates that we have observed is that barbiturates appear to be more soluble in organic solution than isocyanates. Dissociation of an isocyanurate from an aggregate may occur with higher cost of solvation energy and, as a result, be less favored than dissociation of a barbiturate.

The Activation Energy ($\Delta G^{\ddagger}$) for the Processes That Result in the Coalescence of Pairs of Imide Resonances is ~14 kcal/mol. We define chemical exchange of molecules of CA as any process that leads to the coalescence of pairs of imide resonances. Two types of processes are plausible as conceptual models (Scheme 3): a C_2 rotation involving a molecule of CA occurring within an aggregate, and a dissociation–reassociation mechanism that exchanges molecules of CA between aggregates. Attempts to determine whether the process is intra- or intermolecular have been unsuccessful to date. No significant concentration-dependent broadening of the imide resonances is observed over the range of concentration from 2 to 40 mM. This observation is consistent with either type of mechanism, so long as the dissociation of the molecules of CA from the aggregate is rate limiting. Mixing aggregates **1** and **3** results in complete scrambling of the molecules of CA within 30 s (the shortest time which we can accomplish mixing the components). A spectrum having more lines than **1** and **3** alone (due to aggregates having mixtures of types of CA) is observed within the time required for acquisition of a spectrum after mixing.

By knowing the difference in chemical shift ($\delta\nu$) of imide resonances and the coalescence temperature (T_c), approximate rate constants (k_n) can be estimated accord-

(9) This process shown in eq a involves one symmetric isomer (C_3) undergoing degenerate flips of each spoke/melamine to yield three indistinguishable C_1 isomers (C_1a, C_1b, and C_1c). The equilibrium constant for this expression simplifies to that shown in eq b.

$$C_3 \rightleftharpoons {}^1/_3C_1\text{a} + {}^1/_3C_1\text{b} + {}^1/_3C_1\text{c} \quad (a)$$

$$K_{eq} = [C_1\text{a}]^{1/3}[C_1\text{b}]^{1/3}[C_1\text{c}]^{1/3}/[C_3] \quad (b)$$

Because the three C_1 isomers (C_1a, C_1b, and C_1c) are indistinguishable, this expression simplifies to eq c.

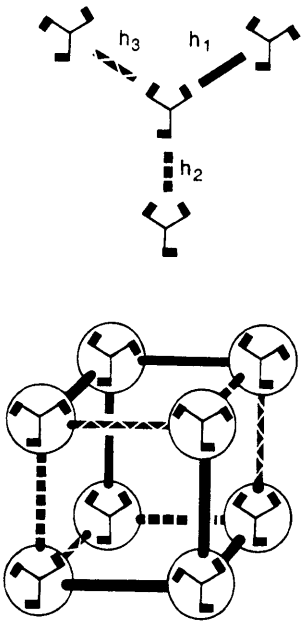
$$K_{eq} = [C_1\text{a}]/[C_3] \quad (c)$$

The value for the equilibrium constant is calculated by comparing the area under the two resonances of the C_3 isomer with $^1/_3$ of area under the six resonances of the C_1 isomer.

(10) We have expanded the data set to include three more isocyanurates and three more barbiturates. This difference in stability appears to be a general trend.

(11) These values were determined from titration curves (pH vs vol 0.1 N NaOH) generated by monitoring pH of solutions (2 mL of water/2 mL of ethanol) of CA (final concentration 150 mM) as 0.1 N NaOH was added. CA **3** was insoluble and **4** was substituted due to the similarity of their structures.

Scheme 4. Rate Scheme for the Isomerization of Hub(M)$_3$[a]



[a] The three different rate processes (rate constants h_1, h_2, and h_3) are indicated with different lines. h_1 describes the interconversion of isomers. Processes h_2 and h_3 are distinct as a result the positions of the adjacent spokes.

ing to eq 2.

$$k_n = \pi(\delta\nu)/\sqrt{2} \qquad (2)$$

The values (for the corresponding T_c) calculated for the different pairs of resonances of the C_3 and C_1 isomers of **1** in CD_2Cl_2 and at the corresponding T_c, these values are between 300 and 700 s^{-1}. In addition, the activation energy for the exchange process ($\Delta G^{\ddagger}$) can be obtained by solving the Eyring equation (eq 3; we assume the transmission coefficient = 1). These values for $\Delta G^{\ddagger}$ are between 13 and 15 kcal/mol.

$$k_n = (\kappa KT/h)e^{-\Delta G^{\ddagger}/RT} \qquad (3)$$

Interconversion of the C_3 and C_1 Isomers Can Be Represented by a Simple Kinetic Scheme. Scheme 4 summarizes the three processes that govern the interconversion of C_3 and C_1 isomers. The rate of interconversion of C_3 and C_1 isomers (h_1) is slow at room temperature: rapid interconversion would result in a single resonance in the imide region since all the molecules of CA would be exposed to more than one hydrogen-bonding environment and show only an averaged signal. The observation of two resonances at room temperature for the C_1 isomer in $CDCl_3$ (Figure 2) indicates that the rates of interconversion of the C_3 and C_1 isomers are not appreciable at this temperature. At higher temperatures (323 K, for **1**) these resonances coalesce. This coalescence may reflect either interconversion of the C_3 and C_1 isomers or exchange of molecules of CA among positions on the isomers. An estimate of the activation energy for this process indicates $\Delta G^{\ddagger} \sim 15$ kcal/mol. We have no solid estimates of the magnitudes of h_2 and h_3 at present.

Conclusions

The region of the ^{1}H NMR spectrum between 13 and 16 ppm of aggregates based on the CA·M lattice contains only resonances due to the N—*H* groups of the isocyanuric or barbituric acid moieties. Characterization of these aggregates is strengthened by analysis of this region. It is diagnostic for the number and symmetry of hydrogen-bonded assemblies present in solution, and the VT ^{1}H NMR spectra shows distinct aggregates assigned C_3 and C_1 symmetries. The ratio of those isomers depends on the solvent only slightly; it is more strongly influenced by the structure of the molecules of CA. The C_3 isomer is favored over the C_1 isomer in all cases.

Experimental Section

General. Synthesis. The syntheses of all compounds and aggregates have been reported.[4]

Sample Preparation. Deuterated solvent obtained from Aldrich (CD_2Cl_2) and the Cambridge Isotope Laboratories ($C_2D_2Cl_4$) were used as received. Chloroform-*d* was Aldrich grade. Spectra were recorded at 10 mM in 0.5 mL of solvent.

Spectrometers. The data were recorded on Bruker AM-500 spectrometers.

Variable-Temperature Spectra. FIDs (32 or 64 scans) were collected using a 90° pulse width (14.5 s) with a short recycling delay[12] (1 s), and an exponential correction (0.2-Hz line broadening) was applied. Temperatures are accurate to within 2° as judged by a Wescon 640D unit. Indistinguishable spectra were generated at a particular temperature on raising the and lowering the temperature.

NOE Spectra. FIDs were collected as a series of two-point 1D experiments (64 scans) with a 6-s decoupler delay and 3-s evolution period using a 90° pulse width. An exponential correction function was applied to the FIDs (0.5–2.0-Hz line broadening). NOEs were obtained by subtraction of the Fourier-transformed spectra.

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(12) Varying the recycling delays from 1 to 30 s did not affect the relative sizes of the lines in the imide region. We selected a short recycler delay to expedite experiments and in order to minimize the temperature variations which occurred during long experiments.